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Review

Pannexin channels in ATP release and beyond: An unexpected rendezvous at the endoplasmic reticulum

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ABSTRACT

The pannexin (Panx) family of proteins, which is co-expressed with connexins (Cxs) in vertebrates, was found to be a new GJ-forming protein family related to invertebrate innexins. During the past ten years, different studies showed that Panxs mainly form hemichannels in the plasma membrane and mediate paracrine signalling by providing a flux pathway for ions such as Ca^{2+}, for ATP and perhaps for other compounds, in response to physiological and pathological stimuli. Although the physiological role of Panxs as a hemichannel was questioned, there is increasing evidence that Panx play a role in vasodilatation, initiation of inflammatory responses, ischemic death of neurons, epilepsy and in tumor suppression. Moreover, it is intriguing that Panxs may also function at the endoplasmic reticulum (ER) as intracellular Ca^{2+}-leak channel and may be involved in ER-related functions. Although the physiological significance and meaning of such Panx-regulated intracellular Ca^{2+} leak requires further exploration, this functional property places Panx at the centre of many physiological and pathophysiological processes, given the fundamental role of intracellular Ca^{2+} homeostasis and dynamics in a plethora of physiological processes. In this review, we therefore want to focus on Panx as channels at the plasma membrane and at the ER membranes with a particular emphasis on the potential implications of the latter in intracellular Ca^{2+} signalling.

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1. Introduction

Cells often respond towards extracellular stimuli, like hormones, growth factors, amino acids and purinergic signalling molecules by the generation of an intracellular Ca^{2+} signal [1]. In many cases, extracellular activation generates intracellular signalling molecules, like inositol 1,4,5-trisphosphate (IP_3) [2]. Elevating intracellular IP_3 levels leads to intracellular Ca^{2+} release that originates from the endoplasmic reticulum (ER) by the opening of IP_3-gated channels (IP_3Rs) [3]. The unique spatiotemporal properties of the Ca^{2+} signal controls a variety of downstream cellular and physiological processes, including gene transcription, cell proliferation, cell survival/death, hormone secretion, enzyme release, actin cytoskeletal contraction, neurotransmitter release and according to synaptic plasticity [2].

In many cases, the cells neither function stochastically nor independently from each other, but rather display a coordinated response towards signalling molecules. This coordination is of utmost importance for the physiological outcome of these processes at the tissue or whole-organ level [4,5], and results from intercellular communication and signalling. The most direct way is via the head-to-head docking of hexameric connexin-based gap junctional channels, allowing the passage of small signalling molecules with a molecular weight of less than 1.5 kDa [6–8]. The activity of these gap junctional channels is tightly regulated by intra- and intermolecular protein interactions and a variety of cellular signalling events, including redox modification and phosphorylation [9]. These gap junctions dictate the coordinated response of connected cells, which can be seen as a chemical and electrical syncitium. The latter is very clear in the heart, where the spreading of an action potential across the atrial and ventricular myocytes is based on connexin-based gap junctional channels [10]. In addition to establishing direct cellular connections, connexins have been shown to act as unapposed "hemichannels" that participate in the release of signalling molecules, like ATP in purinergic signalling [11,12]. Besides connexins, a new family of gap junctional-related channels has been identified, the pannexins (Panx) [12–15]. These channels primarily act as ATP-permeable hemichannels rather than gap junctions [16]. Recent findings also indicated the location of Panx in the ER, thereby forming Ca^{2+}-permeable channels [17,18]. Hence, given their potential role at the plasma membrane and the ER, Panx channels may be at the centre of many signalling processes.

We will first present a general overview of the different mechanisms that control intracellular Ca^{2+} homeostasis. In the next paragraphs we describe the role of Panx channels at the plasma membrane as critical mediators of physiological and pathophysiological signalling. Finally, we discuss the possible role of Panx channels in the ER with an emphasis on their potential role as Ca^{2+}-leak channels and function in neurogenesis.

2. Intracellular Ca^{2+} signals: an overview

In cells ranging from bacteria to highly differentiated eukaryotic cells, ionized calcium (Ca^{2+}) is universal and physiologically important intracellular signalling molecule. In all eukaryotic cells, the cytoplasmic concentration of Ca^{2+} ($[Ca^{2+}]_i$) is tightly controlled by complex interplay between Ca^{2+}-pumps, Ca^{2+}-channels, Ca^{2+}-exchangers and Ca^{2+}-binding proteins [19]. Global or local changes in $[Ca^{2+}]_i$ modulate a wide range of intracellular functions. Muscle contraction, secretion, metabolism, neuronal excitability, cell differentiation, cell proliferation and cell death all depend on Ca^{2+} [20]. The large number of diseases caused by mutations and abnormalities in various proteins involved in the cellular Ca^{2+} regulation also emphasize the importance of Ca^{2+} as an intracellular messenger [21].

The cytoplasmic basal Ca^{2+} activity is maintained at about 100 nM, which is much lower than the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$), which is in the mM range (Fig. 1). Many stimuli can trigger the activation of phospholipase C β/γ (PLC β/γ) and the generation of IP_3, resulting in the release of Ca^{2+} from internal Ca^{2+} stores and a subsequent increase in the cytoplasmic $[Ca^{2+}]$. The increase in $[Ca^{2+}]_i$ depends on the presence and density of cytosolic Ca^{2+}-buffering proteins or Ca^{2+}-buffering organelles such as mitochondria. Furthermore, specialized Ca^{2+} microdomains arise in the close proximity of multi-protein complexes involving Ca^{2+}-channels, which affect local signalling processes or the activity of other proteins, like enzymes and channels [19]. These localized microdomains are established through the formation of Ca^{2+} channel multi-protein complexes, thereby recruiting a variety of effector proteins. Ca^{2+} is delivered from both extracellular space and internal Ca^{2+} stores, such as endoplasmic and sarcoplasmic reticulum (ER and SR), nuclear envelope or Golgi apparatus (Fig. 1). The $[Ca^{2+}]$ in these stores is at least 1000-fold higher than the basal cytoplasmic $[Ca^{2+}]$, creating a high driving force for Ca^{2+} release from the intracellular stores. High

Table 1
Cellular functions of Panx channel.

Functions at the plasma membrane	
Taste bud receptors	Panx1 mediates ATP release to control gustatory afferent nerves during tastant-evoked signalling
Neurons	Panx mediates chemical coupling
Red blood cells	Panx1 mediates ATP release to control microcirculation and micro-environment of the vasculature
Skeletal muscle cells	Panx1 mediates ATP release linked to excitation–transcription regulation during tetanic contractions
Airway epithelial cells	Panx1 mediates ATP release to control mucociliary clearance and maintain proper airway epithelial function during stress
Chondrocytes	Panx1 mediates ATP release (and concomitant decline in intracellular cAMP levels) to control differentiation and proliferation
Keratinocytes	Panx1 causes disorganization, whereas Panx3 channels mediate differentiation
T cells/B cells	Panx1 mediates ATP as co-stimulator for antigenic stimulation
Cells of immune system	Panx1 in "death pore"-complex formation with $P2X_7$
Functions at the ER or other intracellular compartments	
Prostate cancer cells	Panx1 as ER Ca^{2+}-leak channel
Glioma	Panx2 as an inhibitor of cell growth/proliferation
Neuronal progenitor cells	Panx2 neurogenesis and stem-cell like behavior

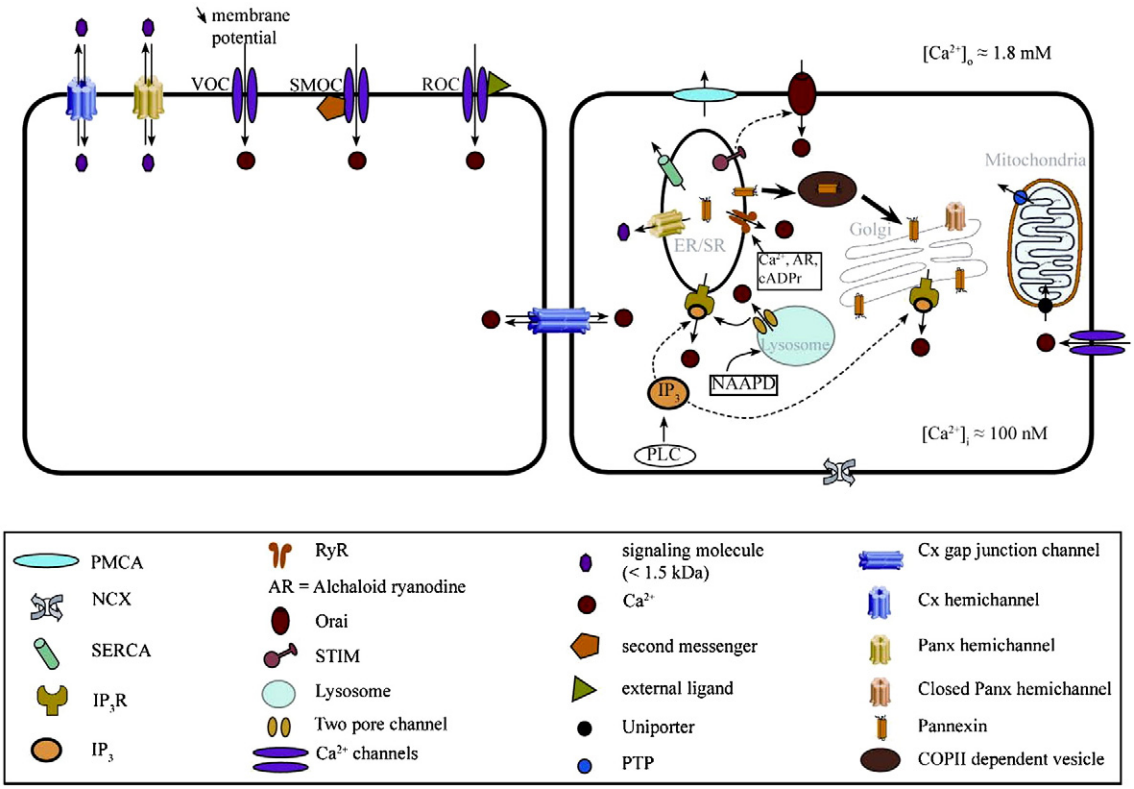

Fig. 1. Ca^{2+}-signalling pathways. Schematic representation of the main players in Ca^{2+} homeostasis and of the different Ca^{2+}-release mechanisms.

cytoplasmic $[Ca^{2+}]$ levels and long-lasting Ca^{2+} increases are toxic [22,23] and may lead to apoptosis and necrosis [24–26]. Therefore, intracellular Ca^{2+} levels are accurately regulated in magnitude, space and time by the intrinsically low permeability of the plasma membrane to ions, and by the activity of channels, pump mechanisms and exchangers in the plasma membrane and in the membranes of the intracellular Ca^{2+} stores [19]. Cells possess an extensive 'Ca^{2+} signalling toolkit' in order to fulfill their physiological functions. Each cell type has a pallet of Ca^{2+}-ON mechanisms, regulating $[Ca^{2+}]_i$ increase, and Ca^{2+}-OFF mechanisms, used to decrease the $[Ca^{2+}]_i$ after Ca^{2+} has carried out its signalling functions [2]. By employing the different components of this pool of Ca^{2+}-handling proteins, cells are able to alter the amplitude, speed, space, and time duration of the Ca^{2+} signal [2]. These mechanisms lead to Ca^{2+} transients that originate in a restricted area of the cell and spread throughout the whole cell as an intracellular Ca^{2+} wave.

Release of Ca^{2+} from intracellular stores occurs in most situations secondary to activation of PLC. Upon PLC activation, IP_3 is formed from phosphatidyl 4,5-bisphosphate (PIP_2) and binds to the cytosolic face of the IP_3 receptor (IP_3R), inducing receptor activation, channel opening and the release of Ca^{2+} from ER Ca^{2+} stores [27]. Another intracellular Ca^{2+}-release channel is the ryanodine receptor (RyR), which is physiologically activated by direct interaction with the dihydropyridine receptor (RyR1 in skeletal muscle) or by Ca^{2+} influx through L-type Ca^{2+} channels (RyR2 in cardiac muscle) (Fig. 1) [28]. Importantly, RyRs also participate in IP_3R-mediated Ca^{2+} signalling via a mechanism known as Ca^{2+}-induced Ca^{2+} release (CICR). Other physiological activators of RyRs include adenosine-based second messengers, like cyclic adenosine diphosphate ribose (cADPr) [29]. Nicotinic acid adenine dinucleotide phosphate (NAADP) is involved in the production of a Ca^{2+} signal via the recently described family of two-pore channels (TPC) [30–32]. This Ca^{2+} signal can be amplified by CICR by recruiting other intracellular Ca^{2+}-release mechanisms [33]. Finally, polycystin-2 has been proposed to operate as a Ca^{2+}-release channel in the ER [34]. The activation mechanism of polycystin-2 is not fully understood but involves Ca^{2+}-induced Ca^{2+} release via a recently identified EF-hand Ca^{2+}-binding site in polycystin-2 [35] and the complex formation with IP_3R [36,37] and RyRs [38].

Besides the presence of intracellular Ca^{2+}-release channels in the ER, which open in response to signalling molecules, like IP_3 and/or Ca^{2+}, one of the most enigmatic processes that control ER Ca^{2+} dynamics is the passive Ca^{2+} leak from the ER. Nevertheless, the molecular nature of the passive Ca^{2+}-leak channel has not yet been fully identified and understood. Many molecular candidates (presenilins [39], polycystin-2 [40–42], translocon [43,44], but see also [45], Bax Inhibitor-1 [46], hypersensitive IP_3Rs activatable by basal IP_3 levels [47,48], caspase-3-cleaved IP_3Rs [49–51] and ER-localized Panx1 channels [18]), have been proposed to account for the passive Ca^{2+}-leak channel, but as yet there is no consensus about the mechanism or function of either of them. On the one hand, the effects of many of these proteins on ER Ca^{2+} or their Ca^{2+}-channel properties were often reported by only one single group or could not be confirmed by other laboratories. On the other hand, in many cases, these proteins interact with the IP_3R and RyR Ca^{2+}-release channels, and may thereby affect their Ca^{2+}-flux properties [36–38,52–54]. It remains therefore difficult to allocate the effects of the candidate proteins on $[Ca^{2+}]_{ER}$ as originating from their own Ca^{2+} permeability rather than from an indirect effect on existing ER Ca^{2+}-release channels.

Activation of Ca^{2+} signalling is rapidly terminated and resting conditions for $[Ca^{2+}]_i$ are restored, by rapid removal of Ca^{2+} from the cytosol. This termination of the Ca^{2+} signal occurs via Ca^{2+} extrusion mechanisms, such as the plasma membrane Ca^{2+}–ATPase (PMCA) and the Na^+/Ca^{2+} exchanger (NCX), and mechanisms refilling the intracellular stores, like sarco-endoplasmic reticulum Ca^{2+} ATPases (SERCAs) (Fig. 1). In addition to the role of Ca^{2+}-buffering proteins, mitochondria modulate agonist-induced Ca^{2+} signals due to the physical association of the ER with mitochondria, known as the mitochondria-associated ER membrane (MAM), allowing highly efficient transmission of Ca^{2+} from

the ER to mitochondria [55,56]. Given the high local [Ca^{2+}] in the ER/mitochondrial microdomains (between 5 and 20 μM), even low-affinity mitochondrial Ca^{2+}-uptake mechanisms can rapidly promote Ca^{2+} flux down its electrochemical gradient. The membrane potential over the inner mitochondrial membrane is highly negative (−180 mV) and is established by the translocation of protons across the inner mitochondrial membrane via respiratory chain complexes (Fig. 1).

In the absence of compensatory Ca^{2+}-influx mechanisms, the ER Ca^{2+} store content would empty over time, leading to ER stress and cell death. Therefore, proper ER Ca^{2+}-store content must be adequately maintained by store-operated Ca^{2+}-influx mechanisms [57]. Recent studies identified Stim proteins as the ER luminal sensor that activates store-operated Ca^{2+} channels, like the newly discovered Orai channels [58–64]. Upon Ca^{2+}-store depletion, Stim oligomerizes and translocates to punctae that are in close proximity with discrete plasma membrane patches, in which plasmalemmal Orai Ca^{2+} channels are recruited by direct or indirect association with Stim1 and become activated, leading to Ca^{2+} influx (Orai, for review [57,65]) (Fig. 1). Besides the role of STIM1/Orai proteins in store-operated Ca^{2+} entry, these proteins have been proposed to be integral parts of the highly Ca^{2+}-selective ARC channels, which are activated by arachidonic acid [66]. ARC channels, whose pentameric architecture is composed of Orai1/Orai3 subunits, are not regulated by store depletion, but require a pool of STIM1 that is constitutively present in the plasma membrane. Other mechanisms that can mediate $[Ca^{2+}]_i$ increases through Ca^{2+} influx involve the opening of plasma membrane Ca^{2+} channels, which are divided into three different classes according to their activation mechanism, the voltage operated Ca^{2+} channels (VOCs), which open following a decrease of membrane potential [67], the second messenger operated channels (SMOCs), which open in response to the binding of a second messenger on the inner surface of the membrane [68] and the receptor operated Ca^{2+} channels (ROCs), also called ligand-gated channels, which open following the binding of an external ligand (Fig. 1) [69].

Besides these plasmalemmal channels, it has become increasingly clear that Pannexins (Panx) can form non-selective large-pore hemichannels in the plasma membrane [70]. Since Panx channels might permeate Ca^{2+} ions and can release signalling molecules that lead to intracellular Ca^{2+} release, it is likely that Panx channels modulate intracellular Ca^{2+} homeostasis. Panxs have a broad expression in the central nervous system (CNS) [71,72]. Panx channels are known to release signalling molecules, like ATP and arachidonic acid derivatives, from neurons and possibly astrocytes [8,73]. In isolated taste cells, Panx1 hemichannels are shown to release ATP and mediate cell–cell communication [74]. This may contribute to novel forms of non-synaptic communication in the CNS, thereby affecting synaptic function, astrocytic Ca^{2+} wave propagation and possibly regulation of vascular tone in the brain. *In vitro* experiments have suggested a possible role for Panx1 channels in ischemic, excitotoxic and ATP-dependent cell death, whereas Panx coupling with purinergic receptors triggers the inflammasome (reviewed in [75]). In this review, we will focus on Panx channels as release channels in the plasma membrane and we will in addition discuss their possible role as Ca^{2+}-leak channels in the ER.

3. Expression, function and localization of Panx channels

In contrast to the 24 members that have been identified in the Cx-protein family (identified by their molecular mass), the Panx family contains only three members (Panx1, Panx2 and Panx3) [14,76]. The Panx-protein family is highly conserved from the worm to mammals [14]. Although Panx channels do not share sequence homology with Cx channels, they display a similar overall structure and permeability properties. Panx channels consist of an N-terminal cytosolic domain, 4 transmembrane domains (TMD) with two extracellular loops between TMD1/TMD2, and TMD3/TMD4, an intracellular loop between TMD2/TMD3, and a C-terminal cytosolic domain [14,17,77]. Multimeric Panx proteins are known to predominantly form large-pore channels in the plasma membrane [13,78]. Different cellular processes, including membrane depolarization and intracellular Ca^{2+} signals, are known to activate and open Panx channels [12]. These channels are permeable for small signalling molecules (<1 kDa), like ATP, thereby playing an important role in autocrine and paracrine purinergic signalling. Among all Panx channels, Panx1 and Panx3 are closely related, while Panx2 is more distant. Panx1 is ubiquitously expressed in many organs, including the eye, thyroid, prostate, kidney and the liver, and a very high Panx1 expression has been found in the central nervous system (hippocampus, neocortex, cerebellum, thalamus and hypothalamus), including hippocampal and neocortical pyramidal neurons, interneurons, motorneurons and purkinje cells [79]. In contrast to the ubiquitously expressed Panx1, Panx2 seems to be mainly expressed in the central nervous system [80,81]. Finally, Panx3 is localized in the skin, osteoblasts and chondrocytes [13,82,83]. Importantly, Panx-expression levels seem to be dynamically regulated by physiological and pathophysiological processes. For instance, although Panx2 is absent in healthy astrocytes, ischemia/reperfusion induces the expression of Panx2 in astrocytes [84]. According to altered mRNA and protein levels upon epileptic or seizure activity [85–91], expression of Panx1 and Panx2 is also shown to be increased as a result of seizure-like activity [92].

Panx1 has been shown to interact with Panx2 and to a lesser extent with Panx3 in a glycosylation-dependent manner [93]. Panx2 strongly interacts with the core and high-mannose species of Panx1, but not with Panx3. All three Panx members are able to traffic to the cell plasma membrane surface and to form functional hemichannels [93]. Efficient plasma membrane targeting of Panx1 channels seems to require glycosylation at N254 [17,77,94]. The delivery of Panxs to the cell surface, their dynamic organization at specific cell-surface microdomains, and their dependency on an intact cytoskeletal network, are beginning to emerge. Notably, all Panx isoforms are not only present in cell-surface clusters, but also displayed intracellular profiles [17,93]. In contrast to the well-studied connexin species Cx43, whose complex network of direct and indirect binding partners that control Cx43-life cycle has been extensively characterized, the identification of the different Panx-binding partners is yet still in its infancy. However, recent reports indicated the association of Panx1 with a protein subunit of the voltage-dependent potassium channel [95], with $P2X_7$ [96,97], and with actin microfilaments, regulating its cell-surface localization and mobility [94] (Fig. 2). Actin directly binds to the C-terminus of Panx1. The interaction of Panx1 and Panx3 with actin microfilaments as well as functional cytoskeletal microfilaments is required for proper Panx1 delivery and dynamics at the cell plasma membrane surface. Importantly, these cytoskeletal interactions seem to stabilize the cell-surface population of Panx1 and Panx3 isoforms [94].

In earlier experiments, Panx1 was found to enable the formation of Ca^{2+}-permeable gap junction channels between adjacent cells, allowing direct intercellular Ca^{2+} diffusion and facilitating intercellular Ca^{2+} wave propagation [13,98–101]. Recently, the link between Panx channels and gap junction channels has been abandoned [72,75,77,102]. Indeed, there were only few reports about Panxs gap junction formation and all reported cases have been in overexpression systems [13,103]. The glycosylation of Panx channels at asparagine residues present at the extracellular site make functional docking of two Panx hemichannels into a intercellular gap junction very unlikely [17,76,77,102]. In addition, Panx channels lack a third extracellular cysteine in the extracellular loops [14,82,104]. Therefore, it is anticipated that unapposed Panx channels have very low affinity for each other, also decreasing the effectiveness of Panx gap junctional channel formation. Several hypotheses were raised to explain the gap junction formation in some rare studies, such as the weakening of the

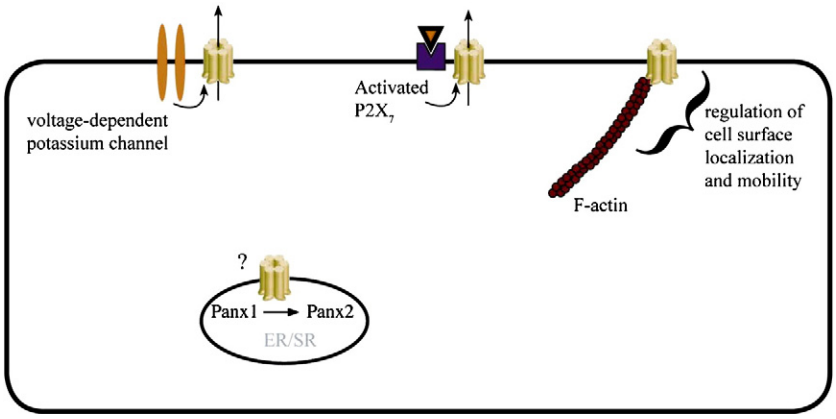

Fig. 2. Different Panx-binding partners and their localization in the cell. Panx1 interacts with a protein subunit of the voltage-dependent potassium channel. A regulatory cross-talk of Panx1 with $P2X_7$ is indicated and interaction of Panx1 with F-actin microfilaments at the carboxyl terminus is shown to regulate its cell-surface localization and mobility. Possible binding partners of Panx1 in the ER are still to be elucidated, but may involve Panx2. In different studies, Panx2 has been observed in intracellular vesicle-like compartments in the perinuclear region.

glycosylation [75], the forced overexpression in oocyte systems and the mixing up of Panx gap junction channels with connexin gap junction channels [105]. Panx expression could indeed influence expression of other proteins, e.g., the connexin expression and hence influence the formation of connexin gap junction channel [105]. Yet, the presence of pannexin gap junctional channels under normal physiological conditions has not been demonstrated, and most studies actually support the assumption that Panxs, in particular Panx1, form single membrane channels [16,17,73,77,82,96,106].

The opening of unapposed pannexin channels in the membrane is triggered by a plethora of physiological triggers, including intracellular Ca^{2+} release, membrane depolarization in the physiological range (−20 mV to +20 mV), mechanical stimulation and $P2X_n$ receptor activation, allowing the release of low-molecular weight signalling molecules, like ATP [13,70,107]. Using the oocyte-expression system, a large unitary conductance of about 500 pS was determined for the fully open state [70]. Although it is tempting to speculate that only Panxs participate in hemichannel-mediated ATP release under physiological conditions, it is clear that also connexin hemichannels can be opened during physiological signalling. For instance, $[Ca^{2+}]_i$ increases in the range of physiologically relevant Ca^{2+} signalling (between 300 nM and 1 μM Ca^{2+}) lead to the opening of Cx32 and CX43 hemichannels [108,109] and thereby can contribute to physiological purinergic signalling [110]. Although it is clear that Panx-hemichannel activation is unaffected by changes in $[Ca^{2+}]_o$ [111], the regulation mechanism of the opening and closure of Panx hemichannels by changes in $[Ca^{2+}]_i$ is not fully understood and might be cell-type dependent. While patch-clamp experiments on Panx1 expressed in *Xenopus* oocytes revealed that increasing $[Ca^{2+}]_i$ above the resting levels (>100 nM) leads to larger Panx1 currents [107], in mammalian cells it has been reported that $[Ca^{2+}]_i$ increases do not trigger Panx1-hemichannel opening or ATP release [112,109]. Additionally, an increase in $[Ca^{2+}]_i$ is not absolutely required for Panx1-hemichannel activation in hippocampal neurons, since the activation of Panx1 channels through NMDARs was independent of the increase in $[Ca^{2+}]_i$, demonstrated by Panx1-hemichannel opening in the presence of intracellular Ca^{2+} buffers [113]. Irrespectively of their activation mechanism, it is however likely that Panx1 hemichannels play an important role in mediating ATP release and intercellular communication. Therefore, opening of Panx1 hemichannels may not only mediate intercellular communication via paracrine purinergic signalling, but also control intracellular Ca^{2+} dynamics by providing a Ca^{2+}-influx pathway independent of the recently identified store-operated Ca^{2+}-influx mechanisms controlled by Stim/Orai.

A role for calmodulin on Panx1 hemichannels can, however, not be ruled out. Although calmodulin-binding sites were not yet identified on Panx1 thus far, calmodulin is an important regulator of Cx50 [114] and Cx32 [115] channel gating, and also interacts with other Cx isoforms, including Cx43 [116].

Also, endogenous "brake" mechanisms that prevent excessive and thus pathophysiological opening of Cx43 hemichannels have been identified [117]. Indeed, the activation of the actin cytoskeleton imposes a strong inhibitory action on Cx43 hemichannels, preventing its opening during high $[Ca^{2+}]_i$ increases (>1 μM) or physiological triggers [117–119]. For instance, thrombin acts on corneal endothelial cells and inhibits Cx43-hemichannel opening in response to mechanical stimulation [118,119].

Panxs are not exclusively localized in the plasma membrane, since Panx1 is also abundantly detected in intracellular organelles such as ER and the Golgi apparatus (Fig. 1) [18,81,103,120]. Panx1 overexpression in human prostate cancer epithelial LNCaP cells, showed accumulation in both the plasma membrane and in the ER [18], implying that post-translational modification and assembly of pannexons share the same route as demonstrated for connexins [121,122]. Recently, it was reported that both Panx1 and Panx3 are co-translationally inserted into the ER and transported by a COPII (coat protein II)-dependent mechanism to the Golgi apparatus, where they are substrates for further glycosylation and editing (Fig. 1) [94]. Panx2 is more abundant in intracellular compartments, except when co-expressed with Panx1, when its cell-surface distribution increases by two-fold [93]. Heteromeric Panx1/Panx2 channels are characterized by compromised channel function, whereas Panx1/Panx3 channels are normal functional channels. Apparently the functional state and cellular distribution of Panxs is regulated by their glycosylation status and interactions among Panx members [93].

4. Signalling by Panx channels located at the plasma membrane

4.1. Physiological signalling

Panx hemichannels can form channels with a large pore, which can conduct molecules smaller than ~1 kDa [73], indicating that Panx channels could be permeable to ions, second messengers and neurotransmitters, such as ATP, IP_3 and amino acids. In ectopic expression systems, and also in normal physiological cell systems, like taste bud receptor cell erythrocytes and in cultured astrocytes, Panx1 directly mediates release of ATP [73,75,78].

The Panx-mediated release of ATP in the extracellular environment can serve as a physiological signal for intercellular communication as well as a noxious signal that promotes cell death. Panx1-mediated ATP release can activate P2Y receptors [107], thereby leading to ATP-induced ATP release. In contrast, ATP can also inhibit the opening of Panx1 channels [123]. This negative feedback of ATP on pannexin channels is

important to prevent a deadly ATP-induced ATP release loop under physiological signalling conditions that would inevitably lead to collapse of energy gradients and cell death. The inhibition of Panx channels by its permeant may provide an important brake on detrimental, excessive Panx-channel opening [124]. In contrast to Cx32 and Cx43, the regulation of Panx-channel activity by intracellular $[Ca^{2+}]_i$ does not display a bell-shaped dependence [108,109,117]. Therefore, the inhibitory mechanisms that turn off channel activity will likely differ between Panx and Cx hemichannels, involving an inhibitory effect of ATP on Panx and Ca^{2+}-dependent inhibition of Cxs through the activation of the actin cytoskeleton-mediated contractility, respectively [108,109,117].

Panx-hemichannel activity has been implicated in different cellular and physiological systems as will be outlined below (Table 1).

4.1.1. Taste bud receptor cells

A prototypical example of physiological Panx1-controlled purinergic signalling occurs in the peripheral taste organ [120,125,126], where the afferent output of gustatory receptor cells exclusively relies on the extracellular release of ATP via a non-vesicular mechanism through Panx1 hemichannels [127]. The binding of sapid molecules to G-protein coupled gustatory receptors in the taste bud receptor (Type II) cells leads to activation of PLCβ2 and IP_3-mediated Ca^{2+} release from intracellular stores. This increase in $[Ca^{2+}]_i$ is the trigger for activation of the TRPM5 channel in the plasma membrane [128]. The opening of the non-selective monovalent cation channel, TRPM5, results in Na^+ influx and membrane depolarization. Physiologically, tastant-evoked intracellular Ca^{2+} release inducing TRPM5-mediated membrane depolarization functions as a trigger for Panx-1-hemichannel opening and ATP release, conveying downstream purinergic ATP-signalling events [128]. On the one hand, ATP will activate the ionotropic $P2X_2/P2X_3$ receptors located on the post-synaptic gustatory nerve fibers of the facial and glossopharyngal nerves [127]. On the other hand, ATP will also act on P2X-receptors located on the adjacent pre-synaptic serotonergic nerve (Type III) cells, which will result in the release of serotonin at the pre-synaptic membrane of these cells [129,130]. Serotonin will activate 5-HT_3 receptors on the post-synaptic gustatory nerve fiber cells, thereby modulating the transmission of taste information from taste buds to gustatory nerves [127]. In addition, multiple forms of feedback exist: i) serotonin exerts negative feedback through 5-HT_{1A} receptors located on the receptor cell (Type II); ii) ATP and its degradation product, ADP, exert positive feedback on the receptor cells through $P2Y_1$ receptors located on the receptor cell to mediate autocrine excitation (Type II) [131].

It is proposed that the frequency of action potentials in the taste cell and the subsequent delivery of ATP in the extracellular environment are proportional to the stimulus intensity, providing a graded taste response. Importantly, ecto-ATPases are abundantly present in the taste buds, allowing rapid and efficient degradation of ATP [132,133]. Therefore, the presence of ATP-release mechanisms, specific ATP receptors and ATP-clearance mechanisms support the role of ATP as a key neurotransmitter linking taste buds and gustatory nerve fibers. Recently, the essential contribution of Panx1 in this cascade was confirmed in intact taste buds [74], supporting a clear physiological role for Panx1-mediated ATP release in the cell-to-cell communication between the taste bud receptor cells and the pre-synaptic cells.

4.1.2. Hippocampus

Since physiological signalling processes can be controlled by Panx1-mediated ATP release, it raises the question whether Panx, which are highly expressed in a variety of neural cells, contribute to ATP release from neurons during physiological brain activity. In this way, Panx channels could mediate non-synaptic chemical coupling and affect synaptic signalling [75]. Besides this chemical coupling, a recent study showed that pyramidal neurons also display a direct electrical coupling [134,135]. However, it remains elusive whether these direct couplings involve pannexin channels. In any case, hemichannels seem to play a role in the release of glutamate from astrocytes [136]. However, the question whether this glutamate release is mediated by Panx remains to be answered. Two recent studies proposed the contribution of Cx43 [137] and/or volume-sensitive channels [138]. Besides signalling processes, an important function of gap junctional-protein family members seem to lie in the coupling of neuronal networks, including astroglial networks, for the exchange of metabolites [139].

4.1.3. Red blood cells

In erythrocytes, which contain high levels of Panx1, electrophysiological and dye-uptake experiments indicated the physiological Panx-hemichannel opening upon depolarization, low oxygen exposure and mechanical stimulation applied as a pressure [107]. Local hypoxic conditions and red blood cell deformation are physiological stimuli for ATP release by erythrocytes, which can trigger ATP-induced ATP release via P2Y receptors located in the erythrocytes as well as in the endothelial layer. It is proposed that intercellular Ca^{2+}-wave propagation, fuelled by ATP-induced ATP release and the involvement of direct gap junctional channels, can lead to the release of NO onto the vascular smooth muscle cell layer, causing its relaxation and leading to vasodilation and increased local blood circulation and oxygen delivery. These effects on the microcirculation would involve an ATP-induced ATP release-signalling wave spreading through the endothelial layer. This is likely to be mediated by the concerted action of Panxs and P2Y receptors [107], given the highest expression level of Panxs in the vasculature of the heart and skeletal muscles. The vascular response to ATP could be amplified by the secretion of cis- and trans-epoxyeicosatrienoic acids (EET) from erythrocytes [140]. EETs are essential components of key vasoregulatory mechanisms and are candidate endothelium-derived hyperpolarizing factors [141]. These vaso-active lipid mediators are metabolized from arachidonic acid and released by ATP-dependent stimulation of erythrocyte P2X7 receptors. The latter action requires functional channels, like CFTR and Panx.

4.1.4. Skeletal muscle cells

Depolarization of skeletal muscle cells (either *in vitro* by electrical stimulation or *in vivo* by opening of nicotinic acetylcholine receptors at the neuromuscular junction) leads to activation of the dihydropyridine receptor and opening of RyR SR Ca^{2+}-release channels, resulting in the contraction of the skeletal muscle. A very recent report shows that ATP is released during physical activity of skeletal muscle cells by a mechanism involving Panx1 hemichannels, which have been proposed to be present in T-tubules of adult skeletal muscle [142]. Extracellularly released ATP activates local, plasma membrane-localized nucleotide receptors: P2X receptors that form ion channels and P2Y receptors that are G-protein coupled receptors. Activation of these receptors provoke a transient increase in $[Ca^{2+}]_i$ with specific kinetic properties. ATP-induced activation of P2X receptors leads to an early, fast increase in $[Ca^{2+}]_i$, which is linked to excitation–contraction coupling, whereas ATP-induced activation of P2Y receptors leads to second, slow and more gradual increase in $[Ca^{2+}]_i$, which is linked to excitation–transcription regulation [143]. Although the fast Ca^{2+} transients obtained by tetanic stimulation mainly are mediated through DHPR-mediated RyR activation, it is clear that ATP modulates the amplitude of these Ca^{2+} signals. Indeed, apyrase reduced the depolarization-evoked Ca^{2+} transient by about 20%. It is speculated that ATP-induced opening of P2X receptors may contribute to supplement the skeletal muscle cells with Ca^{2+} needed to maintain contractions. In contrast, the second and slow increase in $[Ca^{2+}]_i$ was completely dependent on purinergic signalling, as apyrase completely abolished the slow Ca^{2+} transient in response to tetanic depolarizations. Although the role of ATP release for skeletal muscle

physiology urges further exploration, it is clear that extracellular ATP and its receptor targets may play an important role in muscle activity and plasticity. Indeed, prolonged incubations with high [ATP] (500 mM), caused up-regulation of IL-6 and c-fos at the mRNA level [142]. Furthermore, a number of skeletal muscle pathologies have been associated with alterations in extracellular ATP metabolism, sensitivity towards ATP and expression of purinergic receptors [144–147].

4.1.5. Airway epithelial cells

Recently, Panx1 channels have been implicated in the release of ATP from airway epithelial cells in response to hypotonic shock. The physiological role of ATP in this system seems to lie in the control of mucociliary clearance, an important host defense mechanism governed by the airway epithelium [148,149]. The mucociliary clearance is dependent on adequate airway surface liquid volume and the frequency of ciliary beating, both regulated by the release of extracellular ATP. Mucociliary clearance is compromised when paracrine ATP signalling and compensatory cAMP signalling are inhibited [150,151]. Furthermore, using small-volume measurements and cell-attached luciferase assays, extracellular [ATP] near the cell surface was estimated to increase above 1 μM [152], a threshold concentration of ATP believed to be needed for the activation of endogenous P2Y receptors. Importantly, given the observation that Panx1 contributes to about 60% of the total ATP that is released upon hypotonic treatment, it is likely that compromised Panx1 function would bring this concentration below the threshold for P2Y-receptor activation. Hence, it is anticipated that Panx1 plays a crucial role in maintaining proper airway epithelium function during stress conditions.

4.1.6. Chondrocytes

Another physiological role for Panxs seems the regulation of differentiation processes in a variety of cell types. Recently, Panxs have been shown to function in the cartilage, which contain specialized cells, chondrocytes [153]. These cells are not vascularized and depend on diffusion and local signalling processes for their oxygen/energy supply and physiological responses. Pannexin 3 is highly expressed in chondrocytes and regulates chondrocyte differentiation and proliferation. Panx3 attenuated proliferative responses to parathyroid hormone and switched chondrocyte cell fate from proliferation to differentiation. The underlying mechanism involves Panx3-mediated increases in ATP mobilization and decreases in intracellular cAMP levels and downstream cAMP-signalling events.

4.1.7. Keratinocytes

Panx not only regulates the differentiation of chondrocytes, but also modulates the differentiation of keratinocytes [154]. In this case, Panx1 and Panx3 display different roles. In rat epidermal keratinocytes, Panx1 overexpression resulted in a disturbed keratinocyte differentiation and disorganized architecture of the organotypic epidermis, whereas Panx-3 overexpression supported keratinocyte differentiation. However, it remains to be elucidated how the channel function of Panx and related ATP release regulates the differentiation process in these cells.

4.1.8. Cells of the immune system

Purinergic ATP signalling acts as a paracrine messenger in the immune system. A variety of immune cells, including macrophages and T cells, express Panx channels. Importantly, recent studies implicated the release of ATP upon T-cell receptor activation through Panx1 hemichannels. Besides the well-known role of ATP as a "danger signal" in the recruitment of the inflammasome (as will be discussed later), there is growing evidence that ATP plays an important role in the activation of regulatory T cells *in vivo* [155]. Effective activation of T cells by T-cell receptor activation requires the release of ATP by Panx1 hemichannels. The extracellular ATP exerts an autocrine action through the activation of $P2X_7$ receptors and downstream signalling events involving MAPK activation [156]. These local ATP signals may be provided by the opening of Panx1 hemichannels responsible for autocrine signalling processes. Besides these processes, ATP may also induce differentiation of T-helper cells [157], while its degradation product adenosine, may play a role in the suppression of T-cell apoptosis via $A2_A$-receptor activation [158]. Yet, the contribution of Panx1 hemichannels in the latter process has not been elucidated. In summary, the release of extracellular ATP in immune responses is likely to modulate the global functioning of the immune system, including the activation, proliferation, differentiation, maturation and apoptosis of immunologic cells.

4.2. Pathophysiological signalling

Aberrant or prolonged Panx-channel opening would lead to a prolonged inward current, membrane permeabilization and the collapse of ionic, metabolic and bio-energetic gradients.

4.2.1. Epileptiform-like activity in the hippocampus

It has been previously reported that oxygen and glucose deprivation (OGD) lead to neuronal necrosis associated with a collapse of ionic, energy and metabolic gradients [159,160]. OGD provoked opening of large-conductance channels, with the electrophysiological and pharmacological characteristics of Panx hemichannels [161]. Their opening during OGD, even at the neural resting membrane potential, is proposed to contribute to "anoxic depolarizations", a key component of ischemic cell death. The cellular loss of glucose and ATP via these open Panx hemichannels will further impair the recovery of the neuron from the insult. Moreover, aberrant Panx-channel opening has also been observed in pyramidal neurons, where N-methyl-D-aspartate receptor (NMDAR) activation leads to a secondary, prolonged inward current and dye flux that were abolished by using specific Panx1-inhibitory peptides and siRNA against Panx1 [162]. Therefore, it is proposed that Panx1-hemichannel opening during increased endogenous NMDAR activity can trigger epileptiform seizure activity in the hippocampus.

4.2.2. Fibrosis in cardiac myocytes

Besides neuropathophysiological conditions, uncontrolled Panx1-hemichannel opening has been implicated in cardiac fibrosis in response to pressure overload. Upon mechanical stress, cardiac myocytes release ATP and UDP *via* Panx1 hemichannels, leading to stimulation of purinergic receptors and subsequent activation of guanine nucleotide-binding proteins. As a result, induction of the expression of a number of fibrogenic factors (like transforming growth factor-β and connective tissue growth factor) occurs and leads to proliferation of cardiac fibroblasts [163].

4.2.3. Inflammatory responses

High extracellular [ATP] originating from damaged or death cells or prolonged Panx1-hemichannel opening may contribute to inflammatory responses and are considered "danger signals" to the immune system that will lead to cell death if sustained [97,164]. An excellent receiver for these signals is the P2X7-receptor, since this type of P2-receptors requires very high $[ATP]_e$ (>100 μM) for its activation and are not desensitized upon stimulation with high $[ATP]_e$ (>100 μM). Furthermore, there is growing evidence that Panx1 plays a role in the immune system: i) Panx1 channels directly interact with $P2X_7$ receptors, and ii) stimulation of P2X7 by ATP can lead to activation of caspase-1 and secretion of IL-1β within minutes in a Panx-1-dependent pathway [97,165,166]. In cells of the innate immune system (monocytes, macrophages), $P2X_7$ stimulation results in the formation of a non-selective large pore, likely to be the Panx-1 hemichannel itself [165,167]. It is clear that $P2X_7$-mediated Panx-1 activation leads to the assembly of the NLRP3 (Nucleotide-binding domain and leucine-rich repeat receptor containing pyrin domain)

inflammasome complex [164,168,169]. However, it is still under debate whether Panx-1 forms the direct release/entry pathway for signalling molecules like ATP and IL-1β in this process, since Panx-1 shows many characteristics of a chloride channel [112,166]. Currently, it is also unknown what the exact molecular links are between the $P2X_7$/Panx-1 complex and the inflammasome. It appears that the Panx-1 channel forms a pore that allows activation of small pathogen-associated molecular patterns (PAMPs) [169,170]. Recognition of PAMPs as endogenous ligands by cytosolic NOD-like family receptors would enhance the formation of the multimolecular inflammasome complex at which activation of caspase-1 occurs. Interestingly, NLRP3 inflammasome activation followed by IL-1β secretion has recently been found to enable a link between innate (inflammatory) and acquired (cognate) immune responses. IL-1β secretion from dendritic cells is found to be required for T-cell priming [171]. Since this may find its application in anti-cancer immunotherapies, it would be tempting to speculate that increased Panx1 opening could enhance T-cell priming *in vivo*.

Besides the role of Panx1/$P2X_7$ in the activation of the inflammasome in monocytes and macrophages, a recent study reported their contribution to the rapid activation of the inflammasome in neurons and astrocytes [172]. Although the role of K^+ in the mechanism underlying the activation of the inflammasome appears to be different in both systems, it is clear that a similar coupling between Panx1 and P2X7 signalling occurs in the neural system and underlies the rapid cell death that occurs upon P2X7 activation by extracellular ATP.

Another example of the contribution of ATP in inflammatory responses, is the accumulation of extracellular ATP triggering the onset of lung asthmatic airway inflammation [173]. ATP promotes T-helper2 cell sensitization by recruitment and activation of myeloid dendritic cells that present the processed inhaled antigen. While the source of the ATP is yet undefined, Panx-1 channels have been implicated in ATP release from airway epithelial cells and are highly expressed in alveolar macrophages [174].

Cigarette smoke, an inducer of lung emphysema and cause of inflammatory chronic obstructive pulmonary disease (COPD), triggers increased production and release of ATP by neutrophils [175]. While the exact molecular mechanism is not yet clear, ATP release was found not to be caused by cell death. P2-receptors seem to play an important role in the pathogenesis, as smoke induces $P2Y_2$-receptor up-regulation, which is involved in neutrophil recruitment. Blocking of P2-receptors inhibits the inflammation process [176]. Interestingly, Panx-1 hemichannel inhibition is partially successful in preventing Panx-1/$P2X_7$-dependent neutrophil airway influx and cytokine production in a mouse model for lung inflammation and pulmonary fibrosis. These results suggest that ATP is released through the Panx-1 hemichannel after activation by P2X7 interaction [177]. However, it is not unlikely that other hemichannels contribute to intracellular or cell–cell signalling-cascades in the lung, since activated neutrophils are shown to release ATP mainly via Cx43 [178].

Table 2
Questions about ER-localized Panx channels.

Does Panx1 reside in the ER and affect the $[Ca^{2+}]_{ER}$ of cells other than prostate cancer cells, in particular of normal primary cells?
Do other ER-localized Panx isoforms affect $[Ca^{2+}]_{ER}$?
Are Panx channels constitutive passive leak channels or is their activity dynamically regulated by intracellular signalling molecules?
Are conditions known to affect Panx levels or activity associated with changes in $[Ca^{2+}]_{ER}$?
Do high endogenous levels of Panx1 channels, like observed in prostate cancer cells, protect cells against pro-death signals?
Are prostate cancer cells addicted to these high levels of Panx1 channels?
Do other cancer cells elevate Panx1-channel levels in response to oncogenic transformation?

5. Signalling by Panx channels located at the ER and other intracellular compartments

Panxs are believed to preferentially reside in a hemichannel configuration, which is important in paracrine signalling, and can mediate transmembrane transport of Ca^{2+} and ATP in response to physiological and pathological stimuli [17,77,102].

5.1. ER Ca^{2+}-leak channels

In addition to the hemichannel and gap junction function, Panx1 channels were shown to form Ca^{2+}-permeable channels in the ER [18] (Fig. 3 and Table 2). Overexpression of Panx1 channels in prostate cancer cells provoked a severe decrease in the amount of Ca^{2+} that

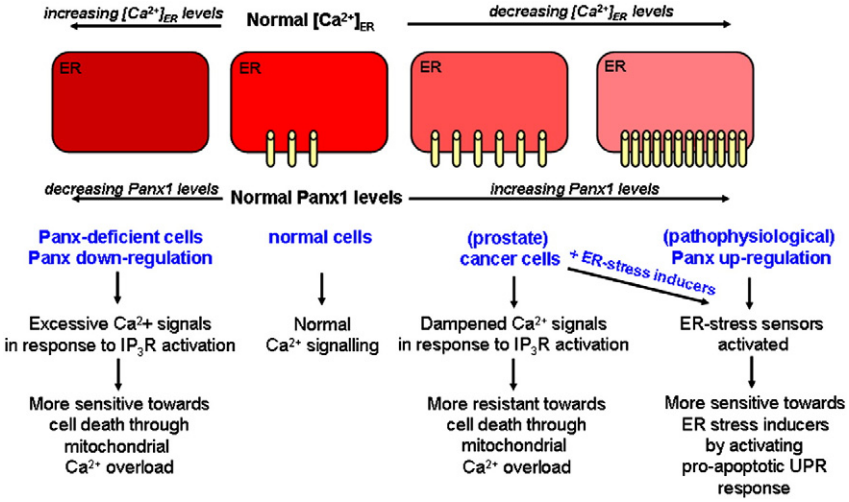

Fig. 3. Panx1 as an ER Ca^{2+}-leak channel. A model for the regulation of $[Ca^{2+}]_{ER}$ by ER-located Panx1 Ca^{2+}-release channels. Under normal conditions, Panx1 levels may contribute to the cellular rheostat, maintaining $[Ca^{2+}]_{ER}$ within a physiological range (~600 uM). Under these conditions, normal IP_3R-mediated Ca^{2+} signalling will occur in response to extracellular signals and ER-stress sensors will be dormant. Panx1 down-regulation can lead to increased $[Ca^{2+}]_{ER}$, which will sensitize the ER towards intracellular IP_3 levels. As a result, excessive Ca^{2+} signals may arise upon agonist-induced Ca^{2+} signalling, leading to mitochondrial Ca^{2+} overload and cell death. In contrast, Panx1 up-regulation, which may occur during pathophysiological conditions, can lead to a severe decrease in $[Ca^{2+}]_{ER}$. The latter has been associated with the activation of the ER-stress sensors and the activation of pro-apoptotic unfolded protein responses. Interestingly, prostate cancer cells seem to exploit Panx1 channels by increasing their levels in the ER membrane. As a result, dampened Ca^{2+} signals will arise in response to IP_3R activation, resulting in an increased resistance towards mitochondrial Ca^{2+}-overload-associated cell death. Hence, increasing Panx1 at the ER may provide an escape route for cancer cells by preventing Ca^{2+}-overload-mediated cell death. Nevertheless, under these conditions, an adaptive unfolded protein response may occur, supporting their survival during decreased $[Ca^{2+}]_{ER}$. As a result, cancer cells may be particularly vulnerable towards chemotherapeutic agents that act as ER-stress inducers.

can be released from the ER upon SERCA inhibition by thapsigargin. These results indicate that increasing Panx1 levels causes a decrease in the steady-state intraluminal $[Ca^{2+}]_{ER}$ [18]. This decrease in $[Ca^{2+}]_{ER}$ was not observed when Cx43 or Cx32 was overexpressed, indicating that i) Panx1-mediated Ca^{2+} leak pathway is probably not an artefact of accumulation of assembled channels in the ER, and ii) the formation of Ca^{2+}-permeable channels in the ER is not a general property of gap junction/hemichannel-forming proteins. Experiments in digitonin-permeabilized LNCaP cells allow a direct assessment of the Ca^{2+}-leak properties of the ER. In these experiments, Panx1 overexpression provoked a dramatic increase in the passive Ca^{2+}-leak rate of the ER. Importantly, this increased Panx1-mediated Ca^{2+}-leak rate was not affected by inhibiting IP_3R/RyR activity. In addition, cells overexpressing Panx1 did not display an increase in proteins previously proposed to affect the passive Ca^{2+} leak from the ER, like the ratio of pro- and anti-apoptotic Bcl-2-family members. The ER-bound Panx1 could be either a pool of unprocessed precursor proteins [81,179] or assembled functional pannexons that serve as ER Ca^{2+}-release channels, thereby facilitating Ca^{2+}-leak from the ER [18]. One caveat of overexpressing Panx1 in prostate cancer cells, which do contain high levels of endogenous Panx1, is the accumulation and entrapment of the ectopic Panx1 channels in the ER, whereas endogenous Panx1 channels may travel to the plasma membrane. Hence, the fact that knock down of endogenous Panx1 channels by siRNA approaches in human prostate cancer cells increased the Ca^{2+} content of the ER by decreasing the efflux rate of Ca^{2+} from the ER, excludes this putative caveat. Moreover, functional and pharmacological characterization of Panx1 channels by electrophysiological approaches revealed that Panx1 channels mediate an outward current that is inhibited by CBX and non-selective chloride channel/anion transporter blockers [112]. Therefore, it is hard to conceive how Panx1 channels that resemble chloride/anion-permeable channels can function as Ca^{2+}-leak channels and this requires further investigation.

Moreover, further research is required to assess whether Panx1 channels reside in the ER and affect ER Ca^{2+} homeostasis in cells other than prostate cancer cells and thus function as *bona fide* Ca^{2+}-leak channels that account for a substantial amount of the passive Ca^{2+} leak of the ER. In particular, the effect of Panx1 knock down in cells endogenously expressing high levels of Panx1 on ER Ca^{2+} should be examined. In addition, it would be exciting to unravel whether the formation of ER Ca^{2+}-leak channels is a general property of the Panx-channel family, using Panx2 and Panx3-overexpressing systems or -knock down approaches. Nevertheless, the finding that Panx1 is at least able to form Ca^{2+}-permeable channels is of utmost importance, since the passive Ca^{2+} leak is one of the most enigmatic processes involved in the regulation of ER Ca^{2+} levels from which its molecular nature and mechanism is very poorly understood.

Moreover, it is not known whether Panx1 channels in the ER are regulated by intracellular signalling molecules, by the ER–Ca^{2+} content or by $[Ca^{2+}]_i$. Since Panx1-hemichannel activity is unaffected by changes in $[Ca^{2+}]_o$ [111], it may be questioned whether $[Ca^{2+}]_{ER}$ affects Panx1 opening. On the other hand, small increases in $[Ca^{2+}]_i$ may stimulate the Ca^{2+} leak from the ER, as Panx-hemichannel activity measured in patch-clamp experiments on Panx1-expressing *Xenopus* oocytes is increased by increasing $[Ca^{2+}]_i$ [107]. Nevertheless, the general role and regulation of Panx channels in the ER needs to be examined in other cell systems.

Furthermore, it has not yet been documented whether pathophysiological conditions, known to increase either Panx1 activity or expression level, like after oxygen and glucose deprivation of pyramidal neurons of the cortex or hippocampus [74,162], affects the Ca^{2+} leak from the ER and thus the steady-state $[Ca^{2+}]_{ER}$ in a Panx1-dependent manner.

Although the function and properties of Panx1 as a Ca^{2+}-leak channels needs to be scrutinized and characterized in other cellular systems under a variety of physiological and pathophysiological conditions, it is important to note that to date the molecular basis of the passive Ca^{2+} leak from the ER generally has been poorly characterized and is a matter of debate [180]. Despite a poor understanding of the molecular mechanisms underlying the passive Ca^{2+} leak, a tight control of the $[Ca^{2+}]_{ER}$ is of utmost importance for physiological Ca^{2+} signalling, cell survival and protein folding [180,181].

On the one hand, increases in Panx1-channel activity or -expression levels may contribute to the development of diseases by decreasing $[Ca^{2+}]_{ER}$ below a critical level. Under those circumstances, ER-resident Ca^{2+}-dependent chaperones, like calreticulin, may function improperly, leading to the accumulation of unfolded proteins and activation of the Unfolded Protein Response [182,183]. Therefore, although normal levels of Panx1 in the ER may contribute to a decrease of the $[Ca^{2+}]_{ER}$ and may be protective against apoptosis, pathophysiological changes in the Panx1 profile of cells (e.g. as a result of neuronal seizure) may lead to activation of severe ER stress and cell death. Consequently, besides the role of Panx1 in ATP-induced cell death as the "large pore" of the P2X7 receptor, changes in Panx1 levels in the ER and its subsequent disturbing effects on ER Ca^{2+} homeostasis may contribute or promote apoptotic cell death involving UPR.

On the other hand, cells lacking Panx1 channels in the ER may display ER Ca^{2+} overload. Under these circumstances, the ER could become hypersensitive towards agonist-induced Ca^{2+} signalling by provoking excessive Ca^{2+} release upon IP_3R activation. Prolonged or exuberant Ca^{2+} signals will perturb mitochondrial function due to the intimate ER/mitochondrial connections. As a result, excessive Ca^{2+} signals may lead to mitochondrial outer membrane permeabilization and cell death. In particular, cells with increased ER Ca^{2+} levels will become particularly sensitive towards pro-apoptotic triggers that promote or synergize with excessive Ca^{2+} signalling. In this perspective, the high levels of Panx1 in the prostate cancer cells may be acting as a protective mechanism against their on-going pro-apoptotic signalling triggered by their oncogenic phenotype by lowering their $[Ca^{2+}]_{ER}$ and thus rendering them more resistant towards the on-going oncogenic signals that would be lethal in normal cells. In this perspective, it would be important to investigate whether prostate cancer cells are addicted to these elevated Panx levels. Hence siRNA approaches targeting Panx1 in prostate cancer cells may elucidate whether Panx1 depletion provokes cell death or sensitizes towards apoptotic inducers. Moreover, the elevated Panx1 levels observed in prostate cancer cells and the subsequent lowering of $[Ca^{2+}]_{ER}$ may render these cells particularly vulnerable towards anti-cancer therapies and drugs that provoke ER stress, like xanthuhomol [184,185]. This approach has been shown to be successful in other cancer malignancies, like chronic lymphocytic leukemia, by provoking ER-stress-induced pro-apoptotic UPR due to their on-going ER-stress signalling.

5.2. Tumor-suppressive inhibition of cell growth

In contrast to Panx1, which has been implicated in a variety of physiological and pathophysiological conditions, the physiological role of Panx2 remains largely undiscovered. Recent studies pointed towards a role for Panx2 in gliomagenesis and oncogenicity [186]. Indeed, by performing a high-throughput cDNA microarray analysis of human brain-tumor samples, Panx2 mRNA levels were found to be reduced in C6 glioma cells in comparison with rat primary astrocytes [83]. The decreased expression of Panx2 was confirmed at the protein level in a variety of human glioma and glioblastoma cell lines [186]. Furthermore, human *Panx2* is located within a chromosomal region often affected in astrocytomas and ependymomas [187–190]. Importantly, stable Panx2-expressing C6 glioma cells displayed an altered, flattened morphology, a reduction in cell proliferation rate through suppression of the cell cycle and decreased saturation density, indicating that Panx2 suppression suppresses the oncogenicity of the C6 glioma cells [186]. These observations were confirmed by

anchorage-independent growth assays and by tumorigenicity assays in immunodeficient mice. Strikingly, these functions of Panx2 seemed independent of its channel function and from its putative action at the plasma membrane. Indeed, the expression pattern of Panx2 revealed an intracellular, cytoplasmic localization with a prominent presence at the perinuclear region in a vesicle-like pattern. In this respect, Lai et al [186] proposed that the long C-terminal tail of Panx2 allows the formation of intracellular protein–protein complexes to mediate its function. Hence, the studies of Prevarskaya on Panx1 [18] and of Naus on Panx2 [186] also implicate that Panx1 and Panx2 have distinct cellular functions and implications for oncogenicity. While increasing Panx1 levels might be a pro-survival strategy of certain types of cancer cells, like prostate cancers, to escape cell death, decreasing Panx2 levels might be pro-proliferative strategy of other types of cancer cells, like brain-related tumors as gliomas, to promote cell growth and oncogenicity.

5.3. Neuronal differentiation

Very recently, Panx2 in the hippocampus has been implicated in postnatal hippocampal neurogenesis [191]. The expression levels, the cellular localization and post-translational modification through S-linked palmitoylation of Panx2 channels are dynamically regulated during the hippocampal neurogenic and differentiation program: first, multipotent neural progenitor cells expresses S-palmitoylated Panx2 that is located in the ER and the Golgi apparatus; later in the program, Panx2 disappears during intermediate progeny, but Panx2 is re-expressed following terminal neuronal differentiation of maturation. Importantly in mature hippocampal granule neurons, Panx2 is present in the plasma membrane, where it forms rod-like structures. Similar observations were made in N2a cells: undifferentiated N2a cells express Panx2 in intracellular compartments, whereas differentiation of N2a leads to relocalization of Panx2 to the plasma membrane. Importantly, knockdown of Panx2 in N2a cells promotes neuronal differentiation. Hence, it was proposed that intracellularly located, S-palmitoylated Panx2 channels play a modulatory role in neurogenesis. Intracellular Panx2 might be important to maintain stem-cell properties of the neural progenitor cells or regulate the timing of their transition into differentiated and mature neurons upon neurogenic stimulation. The exact role of Panx2 at the ER must be elucidated, but a potential interaction between S-palmitoylated Panx2 with Panx1 might contribute to the ER-targeting of Panx1 in neural progenitor cells and to the functional properties of Panx1 channels in the ER.

6. Conclusions

It is clear that Panx fulfill a crucial function as undocked hemichannels, providing a conduit for physiologically relevant signalling molecules, such as ATP. The concerted action of Panx together with P2Y and $P2X_n$ receptors allows a very complex regulation of autocrine and paracrine signalling processes. These seem of relevance in signal-transduction cascades in sensory responses, like in the taste bud receptor cells, in red blood cells to regulate of the microcirculation in the micro-vascular system and in inflammatory responses involving the engagement of $P2X_7$-death receptor complexes. By contrast, aberrant opening of Panx channels seem detrimental in the brain. An ATP-induced inhibition of ATP release by Panx channels therefore provides a possible negative feedback loop, preventing this excessive opening. Notably, novel roles for Panx channels have emerged during the last year. These roles may be Panx-isoform specific and do not necessarily take place at the cell plasma membrane, but may involve other cellular organelles, like the ER and the perinuclear region. In this respect, the role of Panx1 channels as a Ca^{2+}-leak channel to regulate ER Ca^{2+} homeostasis is intriguing. Since the ER plays a central role in the control of intracellular Ca^{2+} signals and protein folding, changes in Panx expression and activity might lead to disturbed ER Ca^{2+} homeostasis, which is a central paradigm in many pathophysiological conditions. Furthermore, other Panx isoforms, like Panx2, might exert important functions at intracellular compartments in the proliferation of brain-tumor cells and differentiation of neural progenitor cells. Therefore, the role of Panx-channel complexes in intracellular compartments during physiological signalling, including the modulation of Ca^{2+}-signalling dynamics and processes, and in response to pathophysiological conditions, including excitotoxicity in neural cells and ischemia, requires further investigations.

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